Laboratory Evaluation of Osteoporosis in Women

Case: A 65 y/o woman steps off of a curb, falls and severe pain develops in her left hip. After a 911 call, EMS brings the woman to the local ED. What is the most likely underlying case of this clinical scenario?

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University of Florida
Departments of Pathology and Pediatrics

Learning objectives: At the conclusion of this presentation, the laboratorian will be able to:
1. Describe the circumstances when the diagnosis of osteoporosis should be considered.
2. List the causes of pathological fractures.
3. Define osteoporosis.
4. Discuss the causes of osteoporosis.
5. Interpret the results of laboratory testing that is conducted during the evaluation of pt’s w/ possible osteoporosis.
6. Interpret bone marker testing results.
7. Understand the controversy that relates to the value of bone marker testing in the evaluation of osteoporosis and its therapy.

Under what clinical circumstances is the diagnosis of osteoporosis considered in women?

Women w/:
(1) Loss in height, kyphosis, other acquired skeletal deformities.
(2) Low BMD on DEXA scan.
(3) Fractures.

Fracture

Pathologic

"Routine" Force > bone strength) ("Exceptional" Force > bone strength)

Stepping off a curb
- > hip fracture

Falling off of a tall ladder
- > hip fracture
What are the causes of pathologic fractures?

Focal bone disease
   Neoplasms
     - Malignant
     - Benign
   Non-neoplastic disease (e.g., bone cyst, osteomyelitis; dysplasias, osteonecrosis)

Generalized bone disease
   Congenital disorders (e.g., dysplasias)
   Acquired disorders

See: http://www.wheelessonline.com/ortho/pathologic_fracture

What are examples of neoplasms that can cause pathologic fractures?

Malignant
   - Bone cancers
     - Malignant fibrous histiocytoma (a.k.a. - fibrous histiocytoma pleomorphic sarcoma)
     - Osteosarcoma
     - Malignant giant cell tumor of bone
   - Non-bone cancers (e.g., multiple myeloma)
   - Metastasis (e.g., breast, lung, kidney, prostate, thyroid)

Benign
   - Benign fibrous histiocytoma
   - Nonossifying fibroma
   - Benign giant cell tumor of bone

What are the causes of pathologic fractures?

Focal bone disease
   Neoplasms
     - Malignant
     - Benign
   Non-neoplastic disease (e.g., bone cyst, osteomyelitis; dysplasias)

Generalized bone disease
   Congenital disorders (e.g., dysplasias)
   Acquired disorders

See: http://www.wheelessonline.com/ortho/pathologic_fracture
What are examples of dysplasias that can cause pathologic fractures?

Dysplasia: congenital, global disorders of abnormal tissue development affecting cartilage and bone.

<table>
<thead>
<tr>
<th>Examples</th>
<th>Etiologies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achondroplasia</td>
<td>GOF mutation in FGFR3</td>
</tr>
<tr>
<td>Thanatophoric dysplasia</td>
<td>&quot;&quot;&quot; &quot;&quot;</td>
</tr>
<tr>
<td>Osteopetrosis</td>
<td>LOF in osteoclasts</td>
</tr>
<tr>
<td>Osteogenesis imperfecta</td>
<td>Mutations in alpha 1 or alpha 2 chains that constitute type 1 collagen</td>
</tr>
<tr>
<td>McCune-Albright syn.</td>
<td>LOF in Gs ATPase</td>
</tr>
</tbody>
</table>

What are the causes of pathologic fractures?

Focal bone disease
- Neoplasms
  - Malignant
  - Benign

Non-neoplastic disease (e.g., bone cyst, osteomyelitis; dysostoses)

Generalized bone disease
- Congenital disorders (e.g., dysplasias)
  - Acquired disorders

Besides osteoporosis, what are examples of generalized, "acquired" disorders that can cause pathologic fractures?

<table>
<thead>
<tr>
<th>Examples</th>
<th>Etiologies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rickets / Osteomalacia</td>
<td>Decr. Vit D activity; hypophosphatemia</td>
</tr>
<tr>
<td>1° hyperparathyroidism</td>
<td>Excess PTH activity</td>
</tr>
<tr>
<td>Renal osteodystrophy</td>
<td>Vit D def., hyperphosphatemia, 2° hyperparathyroidism</td>
</tr>
<tr>
<td>Paget disease of bone</td>
<td>Excess bone turnover</td>
</tr>
<tr>
<td>Humoral hypercalcemia</td>
<td>of Malignancy: PTHrP</td>
</tr>
<tr>
<td>Scurvy</td>
<td>Dietary vitamin C def.</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>Inborn error of sphingomyelin metabol.</td>
</tr>
</tbody>
</table>

What is osteoporosis?

NIH Consensus Statement, Vol 17 (1), March 27-29, 2000

“. . . (A) skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture.”

Eponym: “Porous bone disease”

Consequences of reduced bone strength:

- Loss in height, collapsed vertebra, lumbar lordosis; kyphosis; scoliosis;
- fractures & associated mortality

Most common fractures: Hip, spine, and wrist

What determines bone strength?

Bone density
- gm/area or volume of bone
- Bone mineral density = ~70% of bone strength

Bone quality
- Architecture
- Bone turnover
- Damage accumulation
- Mineralization

Vertebral bodies

Note: Can not measure bone strength directly
Can measure bone density (DEXA scan)
What is the biology of osteoporosis in adults?
Following attainment of maximum adult BMD, over time:

<table>
<thead>
<tr>
<th>Osteoclast activity</th>
<th>Osteoblast activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resorb bone</td>
<td>Build bone</td>
</tr>
</tbody>
</table>

Outcome:
- Reduced bone density (= osteopenia)
- Disordered microarchitecture

Scanning electron microscopy of 3rd lumbar vertebra.

What cells build bone and which cells resorb bone?

<table>
<thead>
<tr>
<th>Function</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoblasts</td>
<td>Build bone</td>
</tr>
<tr>
<td>Osteoclasts</td>
<td>Resorb bone</td>
</tr>
</tbody>
</table>

What is the cycle of normal bone turnover?

*Basic multicellular unit**

* includes osteocytes

Osteoclast (resorption)

OSTEOCLAST (formation)

OSTEOBLAST (formation)
What regulates osteoclasts?

Stromal (osteoprogenitor) cell

Interruption PTH

Osteoblast

HSC

M-CSF

OPG

RANK

RANKL

Osteoclast

precursor

Precursor

fusion

Resting osteoclast

**NOTE:** Normal pulses of PTH ("intermittent" secretion) from the parathyroid gland stimulate the development of osteoblasts from stromal cells.

Stimulated osteoblasts secrete macrophage colony-stimulating factor (M-CSF). This stimulates the replication of macrophages into osteoclast precursors.

Osteoblasts also express on their surface osteoclast differentiating factor (ODF) which stimulate osteoclast precursors differentiation into osteoclasts. ODF is also known as receptor activator of NF kappaB ligand (RANKL) and osteoprotegerin-ligand (OPGL).

Osteoprotegerin (OPG), secreted by osteoblasts, antagonizes the effects of RANKL. OPG serves as a soluble decoy receptor for RANKL antagonizing RANKL’s ability to bind to its receptor which is RANK on osteoclast precursors.

Therefore: Osteoblasts regulate osteoclasts

[Diagram showing inhibition and stimulation of osteoclast activation, competition, and transformation from osteoclast precursor to resting osteoclast]
Osteoprotegerin

RANKL = receptor activator of NF-kappaB ligand
- member of TNF superfamily of ligands & receptors
- essential for: osteoclast differentiation, activation, survival
- also expressed on: marrow stromal cells & activated T cells

RANK = receptor activator of NF-kappaB

OSTEOBLAST REGULATION OF OSTEOCLASTS

How do osteoclasts resorb bone?

Attachment to bone via integrins (alpha,beta3) on foot-like podosomes that contain actin

Active bone resorption

Integrins associate w/:
- osteopontin
- vitronectin
  (produce tight seal)

Basolateral membrane
Resorptive surface
w/ ruffled boarder

Mineral content of bone: hydroxyapatite
(Ca$_3$(PO$_4$)$_2$,Ca(OH)$_2$)

Released products activate osteoblasts

Bicarbonate-chloride exchanger

OSTEOCLAST

MINERALIZED BONE

HCO$_3^-$

Cl$^-$

Integrins (alpha,beta3)

H$_2$O + CO$_2$ → H$^+$ + HCO$_3^-$

Cytoplasmic lumen or subosteoclastic compartment (a.k.a. – resorption pit)

Cl$^-$ channel

N$^+$-ATPase pump

pH ≤ 4.5

H$^+$ → Cathepsin K & MMP-9

Cl$^-$

HCO$_3^-$
**Definitions:**

Transcytosis: A mechanism for transcellular transport in which a cell encloses extracellular material in an invagination of the cell membrane to form a vesicle (endocytosis), then moves the vesicle across the cell to eject the material through the opposite cell membrane by the reverse process (exocytosis).

ALSO: The transport mechanism by which most proteins reach the Golgi apparatus or the plasma membrane;
- the vesicles targeted toward lysosomes and secretory storage granules appear to be coated with clathrin.

Syn: cytopempsis, vesicular transport.

Where do osteocytes come from?

Osteoblasts form new bone where bone was eroded by osteoclasts; osteoblasts become entrapped in bone to become osteocytes.
What maintains bone mass?
Nutrition: dietary Ca++ intake and absorption (Vit D)*
Exercise & Gravity: load bearing (fosters bone remodeling)
Sex steroids
   E2 (women and men)
   Testosterone (men)
Growth hormone
Thyroid hormone

What causes osteoporosis?
Complex: multifactorial:
Environment (+) Genetics (+) Aging
↓  ↓  ↓  ↓
Environment:  Genes  Aging:
Vitamin D & Ca++ intake  Female (estrogen deficiency in females
General nutrition  Male (androgen deficiency in males
Exposure to sunlight  Race (risk: W > H > AA)
Body weight  Gene polymorphisms
Skeletal trauma  Malabsorption
Current smoking

What environmental factors contribute to osteoporosis?
Environment (+) Genetics (+) Aging
↓
Environment:  Incr. risk w:
Vitamin D & Ca++ intake  Low intake
General nutrition  Poor nutrition
Exposure to sunlight  Lack of exposure
Body weight  Low body weight
Skeletal trauma  History of previous fracture
Current smoking  Chronic inflammation
Drugs  Glucocorticoids (>3 mo),

* Need: functional intestine & 1,25(OH)2D, 1,25(OH)2D is a function of 25(OH)2D, PTH, E2, and insulin

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   E2 (women and men)
   Testosterone (men)
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Current smoking  Chronic inflammation
Drugs  Glucocorticoids (>3 mo),
What genetic factors contribute to osteoporosis?
Environment (+) Genetics (+) Aging

Genetics:
- Female gender
- Race
- FHx of osteoporosis
- FHx of fractures
- Gene polymorphisms

Comment:
- Risk: $♀ > ♂$
- W > H > AA
- Vitamin D receptor, LRP5/6
- (+) => 80 other genes

How does aging contribute to osteoporosis?
Environment (+) Genetics (+) Aging

Aging:
- Estrogen deficiency
- Androgen deficiency
- Chronic disease
- Malnutrition
- Malabsorption

Comment:
- Menopause
- Andropause
- Cytokine-induced osteoclast activation
- Impaired appetite w/ aging
- Impaired absorption w/ aging

Abstract
Osteoporosis, the most common type of bone disease worldwide, is clinically characterized by low bone mineral density (BMD) and increased susceptibility to fractures. Multiple genetic and environmental factors and gene-environment interactions have been implicated in its pathogenesis. Osteoporosis has strong genetic determination, with the heritability of BMD estimated to be as high as 60%. More than 80 genes or genetic variants have been implicated in risk of osteoporosis by hypothesis-free genome-wide studies. However, these genes or genetic variants can only explain a small portion of BMD variation, suggesting that many other genes or genetic variants underlying osteoporosis risk await discovery. Here, we review recent progress in genome-wide studies of osteoporosis and discuss their implications for medicine and the major challenges in the field.
What causes osteoporosis?
Complex: multifactorial:

<table>
<thead>
<tr>
<th>Environment (+)</th>
<th>Genetics (+)</th>
<th>Aging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D &amp; Ca++ intake</td>
<td>Estrogen deficiency in females</td>
<td>Androgen deficiency in males</td>
</tr>
<tr>
<td>General nutrition</td>
<td>Aging: osteoclast activation</td>
<td>Malnutrition / malabsorption</td>
</tr>
<tr>
<td>Exposure to sunlight</td>
<td>Body weight</td>
<td>Skeletal trauma</td>
</tr>
<tr>
<td>Gravity (loading)</td>
<td>Genetics:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female gender (risk ♀ &gt; ♂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Race (risk: W &gt; H &gt; AA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>vitamin D receptor polymorphisms</td>
<td>LRP5/6 polymorphisms, etc.</td>
</tr>
</tbody>
</table>

What are the laboratory findings in osteoporosis?

<table>
<thead>
<tr>
<th>Ca++</th>
<th>PO₄</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within reference interval</td>
<td>Within reference interval</td>
<td>Within reference interval</td>
</tr>
</tbody>
</table>

Conclusion:
There is NO diagnostic lab "signature" of osteoporosis!

Why should laboratory testing be performed in cases of suspected osteoporosis?
Primary osteoporosis is a disease of exclusion!
Must: r/o other causes of orthopedic dis./osteopenia/fx’s
Differential diagnosis of ~common and/or important disorders that can present +/- similar to osteoporosis or may co-exist w/ osteoporosis:

- Hyperparathyroidism
- Osteomalacia
- Renal osteodystrophy
- Paget disease of bone
- Osteogenesis imperfecta
- Scurvy

Lab: Major Dx role
Lab: Minor Dx role
X: gene testing
How can hyperparathyroidism be diagnosed in women with orthopedic disease, osteopenia and/or fractures?

**Result**
- Ca\(^{++}\) Increased
- PO\(_{4}^{-}\) Decreased (or low normal)
- ALP Increased (PTH -> osteoblast -> ALP)

**Next diagnostic steps:**
- Always: r/o renal disease (w/ untx'ed CRF: +/- incr. PO\(_{4}^{-}\))
- Confirm hypercalcemia: iCa\(^{++}\) (or alb): r/o dehydration
- Measure: PTH (WNL or incr. = 1° hyperparathyroidism)

How can osteomalacia be diagnosed in women with orthopedic disease, osteopenia and/or fractures?

**Result**
- Ca\(^{++}\) Usually: lower range of reference interval
- PO\(_{4}^{-}\) Decreased (Incr. PTH - > hyperphosphaturia -> hypophosphatemia)
- ALP Increased (PTH -> osteoblast - > ALP)

**Next diagnostic steps:** (r/o renal disease)
- Measure:
  - PTH (incr. = 2° hyperparathyroidism)
  - 25-OHD (decr. w/ vit D def.)
- Many rare forms: hepatic rickets, VDDR, VDRR, etc.
How can renal osteodystrophy be diagnosed in women with orthopedic disease, osteopenia and/or fractures?

**Ca**++ Result
Within reference interval

**PO4**— Result
Increased (PO4— retention)

**ALP** Result
Increased (PTH -> osteoblast -> ALP)

**Cr** Result
Increased (decr. eGFR, CrCl)

Next diagnostic steps:
Measure: PTH (incr. = 2° hyperparathyroidism)

---

How can Paget disease of bone (osteitis deformans) be diagnosed in women with orthopedic disease and/or fractures?

**Ca**++ Result
Within reference interval

**PO4**— Result
Within reference interval

**ALP** Result
Increased (cytokine? -> osteoblast -> ALP)

Paget disease of bone: Not a lab diagnosis!

Physical examination: deformities; hearing, CN problems

Radiologic findings

Remember: r/o renal disease

---

How can osteogenesis imperfecta be diagnosed in women with orthopedic disease, osteopenia and/or fractures?

**Ca**++ Result
Within reference interval

**PO4**— Result
Within reference interval

**ALP** Result
Increased w/ fracture

Osteogenesis imperfecta: *Not a chem. lab diagnosis!*

Brittle bones that fracture
+- Hearing loss; blue sclera; dental problems
Many disorders w/ variable severity

Radiologic findings: Extremely helpful; Gene testing avail.
How can scurvy be diagnosed in women with orthopedic disease and/or fractures?

<table>
<thead>
<tr>
<th>Result</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca**</td>
<td>Within reference interval</td>
</tr>
<tr>
<td>PO4---</td>
<td>Within reference interval</td>
</tr>
<tr>
<td>ALP</td>
<td>Increased w/ fracture</td>
</tr>
</tbody>
</table>

Scurvy: ~Not a chem. lab diagnosis (unless vit C is measured)! (Dietary vit C def.)

4 H’s: Hemorrhage, hyperkeratosis, hypochondriasis*, and hematologic abnormalities (anemia from blood loss).

*an overwhelming fear that you have a serious disease, even though health care providers can find no evidence of illness.

Plain film

This is the preferred initial examination.

head, neck and spine
mandible
sphenoid sinuses
vertebral bodies
vertebral compression fractures
spinal vertebrae
paraspinally
chest
shoulder or elbow (neck of femur)
pelvis
acetabular protrusion
general
severe osteoporosis
deformed, gracile (over-tubulated) bones
cortical thinning
perfusion defects
bone formation abnormalities (formation, absence, or formation)
papillaronic calcification: the metaphyses and epiphyses exhibit numerous scalloped radiolucent areas with sclerotic margins
anterior stripe signs: cyclic bisphosphonate treatment produces sclerotic growth recovery lines in the long bones
exuberant callus formation
formation of pseudarthrosis at sites of healing fractures

Source: [http://radiopaedia.org/articles/osteogenesis-imperfecta-1](http://radiopaedia.org/articles/osteogenesis-imperfecta-1)

How can bone formation be evaluated through laboratory testing?

<table>
<thead>
<tr>
<th>Marker</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone alkaline phosphatase (BAP)</td>
<td>Produced by osteoblasts</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>Produced by osteoblasts Non-collagenous bone matrix protein</td>
</tr>
<tr>
<td>Procollagen N-terminal propeptide (P-PINP)</td>
<td>Formed by conversion of procollagen --&gt; collagen</td>
</tr>
</tbody>
</table>
How is procollagen N-terminal propeptide (s-PINP) formed?

Procollagen

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>N-telopeptides</td>
<td>Bone collagen degradation product</td>
</tr>
<tr>
<td>Urine NTx</td>
<td>30-40% decr. from NTx baseline post-3 mos tx = typical response to anti-resorptive therapy.*</td>
</tr>
<tr>
<td>Serum NTx</td>
<td>Target value for tx'ed post-menopausal women = premenopausal reference interval.**</td>
</tr>
<tr>
<td>Urine C-telopeptides (CTx)</td>
<td>Bone collagen degradation product</td>
</tr>
</tbody>
</table>


How are NTx and CTx formed?
One NTx assay: ex: MoAb 1H11 to this octapeptide NTx from N-terminus of α2 collagen protein

C-Telopeptide: released with collagen degradation

Collagen type I

Type 1 collagen

N-telopeptides

α1(1): SAGDFSFLPQPQKEKAHDGRRYYRA

CTx, CrossLaps™ assay: MoAb to this octapeptide

How can bone turnover be evaluated through laboratory testing?

<table>
<thead>
<tr>
<th>Markers of bone degradation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine deoxypyridinoline (DPD)</td>
<td>Bone collagen degradation product Target value for tx’ed post-menopausal women = premenopausal reference interval.*</td>
</tr>
<tr>
<td>Urine pyridinoline</td>
<td>Bone collagen degradation product</td>
</tr>
</tbody>
</table>

* [http://ltd.aruplab.com/Tests/Pub/89185213](http://ltd.aruplab.com/Tests/Pub/89185213)
How are deoxypyridinoline and pyridinoline formed?

**Pyridinoline**

**Deoxypyridinoline**

(more bone-specific)

Pyridinium crosslinks: either:
- pyridinoline or deoxypyridinoline

Released w/ collagen breakdown

How can bone turnover be evaluated through laboratory testing?

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Tartrate-resistant acid phosphatase</td>
<td>Produced by osteoclasts - reflect bone resorption (TRACP5b)</td>
</tr>
</tbody>
</table>
Limited utility of tartrate-resistant acid phosphatase isoform 5b (TRACP5b) in assessing response to therapy in osteoporosis.

Brady JJ1, Crowley RK, Murray BF, Kilbane MT, O'Keane M, McKenna MJ.

Abstract

BACKGROUND: Tartrate-resistant acid phosphatase isoform 5b (TRACP5b) is a serum bone resorption marker. Our aim was to investigate its utility in monitoring metabolic bone disease.

METHODS: Serum TRACP5b, C-terminal cross-linking telopeptide of type I collagen, urine N-terminal cross-linking telopeptide of type I collagen and free deoxypyridinoline were measured pre- and post-treatment with a parathyroid hormone analogue [PTH (1-34)] (n = 14) or a bisphosphonate (N-BP) (n = 8). TRACP5b, bone alkaline phosphatase (bone ALP), 25-hydroxyvitamin D (25OHD) and parathyroid hormone (PTH) were measured in 100 osteoporosis patients on prolonged bisphosphonate therapy.

RESULTS: Changes in TRACP5b were smaller in magnitude but mimicked those of other bone resorption markers. Absolute changes in TRACP5b and the other resorption markers correlated significantly (p < 0.001). In patients on long-term bisphosphonates, TRACP5b and bone ALP levels were not suppressed. Vitamin D status was consistent with the level of supplementation.

CONCLUSION: TRACP5b has limited utility as a single marker of metabolic bone disease treatment.

Tartrate-resistant acid phosphatase 5b (TRACP 5b) as a marker of bone resorption.

Halleen JM1, Tiitinen SL, Ylipahkala H, Fagerlund KM, Väänänen HK.

Abstract

Tartrate-resistant acid phosphatase (TRACP) is an enzyme that is expressed in high amounts by bone resorbing osteoclasts, inflammatory macrophages and dendritic cells. Two forms of TRACP circulate in human blood, TRACP 5a derived from macrophages and dendritic cells, and TRACP 5b derived from osteoclasts. Recent data have demonstrated the utility of TRACP 5b as a marker of osteoclast number and bone resorption, and serum TRACP 5b as a marker of inflammatory conditions. This review summarizes the scientific knowledge on the role of TRACP in osteoclastic bone resorption, the mechanism of TRACP 5b generation in osteoclasts and its secretion into the blood circulation, the methodology of measuring TRACP 5b and guidelines for the use of TRACP 5b as a resorption marker, and characteristics of TRACP 5b compared to other currently used bone turnover markers.

What are other novel bone markers?

<table>
<thead>
<tr>
<th>Marker</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclerostin</td>
<td>Produced by osteocytes - negative regulator of bone mass</td>
</tr>
<tr>
<td>Inter alpha-trypsin-inhibitor heavy chain H4 precursor (ITIH4)</td>
<td>Serum-borne reflection of the increased osteoclast activity</td>
</tr>
</tbody>
</table>
### What are other novel bone markers?

<table>
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<tr>
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<th>Comment</th>
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</thead>
<tbody>
<tr>
<td>Undercarboxylated osteocalcin (ucOC)</td>
<td>Used to assess the vitamin K status in bone metabolism</td>
</tr>
<tr>
<td>Serum N-terminal midfragment Osteocalcin</td>
<td>Stable fragment of osteocalcin</td>
</tr>
<tr>
<td>Bone sialoprotein</td>
<td>Produced by osteoblasts - Fn (?): protein nucleator of hydroxyapatite crystal formation</td>
</tr>
</tbody>
</table>

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<tbody>
<tr>
<td>Osteoprotegerin (OPG)</td>
<td>Produced by osteoblasts - Decoy receptor for RANKL</td>
</tr>
<tr>
<td>N-terminal propeptide of the C-type natriuretic peptide (NT-proCNP)</td>
<td>Serum concentrations correlate with bone cortical area and endosteal circumference</td>
</tr>
</tbody>
</table>
Bone-forming cells originate from distinct embryological layers, mesoderm (skeletal and appendicular bones) and ectoderm (precursor of neural crest cells, which mainly form facial bones). These cells will develop bones by two principal mechanisms: intramembranous and endochondral ossification. In both cases, condensation of multipotent mesenchymal cells occurs, at the site of the future bone, which differentiate into bone and cartilage-forming cells. During long bone development, an initial cartilaginous template is formed and replaced by bone in a coordinated and ordered process involving chondrocyte proliferation and maturation, vascular invasion, recruitment of adult stem cells and interminar remodeling of cartilage and bone matrix. Matrix metalloproteinases (MMPs) are the major enzymes responsible for clearing structural components of the extracellular matrix (ECM), as well as for degrading extracellular matrix molecules generated in several biologic events, such as development, tissue remodeling and homeostasis. Since the discovery of collagenase in bone cells, more than half of the MMP members have been detected in bone tissues under both physiological and pathological conditions. Pivotal functions of MMPs during development and bone regeneration have been revealed by knock-out mouse models, such as chondrocyte proliferation and differentiation, chondrocyte recruitment and function, bone modeling, coupling of bone resorption and formation (bone remodeling), osteoblast recruitment and survival, angiogenesis, osteocyte viability and function, and cartilage degradation. This review describes an overview of the principal properties of MMPs and their inhibitors (TIMPs and RECK), provides an up-date on their known functions in bone development and remodeling and discusses their potential application in Bone Bioengineering.

Osteoprotegerin (OPG) is an essential secreted protein in bone turnover due to its role as a decoy receptor for the Receptor Activator of Nuclear Factor-κB ligand (RANKL) in the osteoclasts, thus inhibiting their differentiation. However, there are additional ligands of OPG that confer various biological functions. OPG can promote cell survival, cell proliferation and facilitates migration by binding TNF-related apoptosis inducing ligand (TRAIL), glycosaminoglycans or proteoglycans. A large number of in vitro, pre-clinical and clinical studies provide evidence of OPG involvement in resorptive, bone, immune and tumor biology. This review describes an overview of the different OPG signals regulating the biological functions.

Bone turnover provide a means of evaluating skeletal dynamics that complements static measurements of BMD by products in the blood or urine, lack both sensitivity and specificity as a reliable diagnostic tool. As a result, improved tests are needed to augment the use of BMD measurements as the principle diagnostic modality. In this study, the serum proteome of 58 postmenopausal women with high or low normal bone turnover (training set) was analyzed by surface enhanced laser-desorption/ionization time-of-flight mass spectrometry, and the diagnostic fingerprint was identified using a variety of statistical and machine learning tools. The diagnostic fingerprint was validated in a separate blinded set, consisting of 59 unselected postmenopausal women (validation set). The diagnostic proteomics fingerprint was used to identify a subset of 9 serum proteins that were able to differentiate between postmenopausal patients with high or low bone turnover with high specificity and 100% specificity. Additionally, the individual protein peaks were also significantly correlated with BMD measurements in these patients. Four of the major discriminatory peaks in the diagnostic profile were identified as fragments of interalpha-trypsin-inhibitor heavy chain H4 precursor (ITIH4), a plasma kallikrein-sensitive glycoprotein that is a component of the host response system. These data suggest that these serum protein fragments are the serum-borne reflection of the increased osteoclast activity, leading to the increased bone turnover that is associated with decreasing BMD and presumably an increased risk of fracture. In conjunction with the identification of the individual proteins, this protein fingerprint may provide a novel approach to evaluate high bone turnover states.

Importance of vitamin K has been suggested to maintain and improve bone strength. The serum concentration of undercarboxylated osteocalcin (ucOC) was used to assess the vitamin K status in bone metabolism. The undercarboxylated osteocalcin (ucOC) is utilized to assess the vitamin K status in bone metabolism. The measurement of ucOC would be useful to select the patients who require vitamin K treatment.
BACKGROUND/AIM:
Females with anorexia nervosa (AN) are often affected by osteoporosis. The study intends to investigate the association between serum levels of the N-terminal propeptide of the C-type natriuretic peptide (NT-proCNP) and bone development in anorexic females.

SUBJECTS AND METHODS:
In a catamnestic visit, 21 females, formerly treated for AN, were assessed for the presence of eating disorders and evaluated for bone parameters of the distal radius (4% site) with peripheral quantitative computed tomography (pQCT), for maximal isometric grip force (MIGF) and for NT-proCNP serum levels.

RESULTS:
The 9 females with a persistent eating disorder had lower height and weight than the recovered girls. NT-proCNP was correlated with the cortical area (r = 0.521), the endosteal circumference (CE, r = -0.468) and the ratio of MIGF to cross-sectional bone area (r = 0.434). CE explained 40% of the variance of NT-proCNP in females with persistent eating disorders, but was not associated with NT-proCNP in recovered girls (p = 0.691). The association between CE and NT-proCNP was not existent when the correlation was controlled for the duration of amenorrhea and the supplemented cumulative dose of ethinylestradiol (p = 0.275).

CONCLUSION:
NT-proCNP reflects metaphyseal inwaisting which is modified by estrogens and the pressure on the growth plate.

What is the value of bone marker testing in the evaluation of osteoporosis and its therapy?

“BTMs (bone turnover markers) are widely used in bone research including therapeutic trials of new medications for osteoporosis and other bone disease. Whilst further data are awaited to confirm their utility in the clinical management of osteoporosis, they are currently used in specialist clinical practices, especially in monitoring treatment.”


Summary
Osteoporosis is NOT a biochemical diagnosis

Laboratory testing to: r/o:
- Hyperparathyroidism
- Osteomalacia
- Renal osteodystrophy

Clinical/radiologic evaluation for:
- Paget disease of bone
- Osteogenesis imperfecta

Bone turnover markers: clinical value?
Hypophosphatasia

Pathophysiology

Mild Hypophosphatasia

In infants, symptoms are usually present at birth or shortly thereafter. Children may experience bone pain, delayed bone mineralization, and failure to thrive. The condition is often diagnosed after the child begins to walk and experiences increased bone pain.

Severe Hypophosphatasia

In severe cases, symptoms may include bone pain, rickets, and osteosclerosis. These cases are typically diagnosed in infancy.

Inheritance

The condition is inherited in an autosomal recessive pattern. This means that a person needs to inherit two copies of the mutant gene, one from each parent, to have the condition. Each parent of an affected person has one copy of the mutated gene and one copy of the normal gene.

The Hypophosphatasia Gene

The ALPL gene, located on chromosome 1p36, encodes an enzyme called alkaline phosphatase (ALP). Mutations in the ALPL gene result in a deficiency of ALP, which affects bone and tooth development.

Signs and Symptoms

- Bone pain
- Delayed bone mineralization
- Osteosclerosis
- Rickets
- Dental abnormalities
- Hearing loss
- Ocular abnormalities
- Immunodeficiency
- Growth retardation
- Respiratory issues
- Renal failure

Diagnosis

A diagnosis of hypophosphatasia can be made through a combination of clinical symptoms, biochemical testing, and genetic analysis. Bone biopsy and radiographs may also be used to confirm the diagnosis.

Management

Treatment for hypophosphatasia includes nutritional support, physical therapy, and supplements. In severe cases, bone transplants and gene therapy may be considered.

Prognosis

The prognosis for hypophosphatasia varies depending on the severity of the condition. Cases with milder forms may have a better prognosis, while severe cases can lead to severe disability and even death.

Source: